FLAVONOID DIVERSITY IN RELATION TO SYSTEMATICS AND EVOLUTION OF THE TARWEEDS[1,2]

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ABSTRACT

The tarweeds produce a diverse array of flavonoids, including glycosylated compounds and methylated aglycones. Extra hydroxylation at positions 6 and/or 8, with frequent O-methylation at these and other positions, is prevalent. This structural diversity coupled with the variety of classes of flavonoids accumulated (flavanones, dihydroflavonols, flavones, flavonols) provides a rich source of characters for study of relationships within and among genera. The distribution of flavonoid aglycones within *Dubautia* is consistent with morphological, cytogenetic, electrophoretic, and phytogeographic evidence bearing on the evolution of its taxa. Flavonoids have also provided a means of assessing the dynamics of hybridization in *Dubautia*. In *Hemizonia*, multivariate analyses of flavonoid arrays have corroborated the view, based on cytogenetic and morphological criteria, that each perennial taxon is independently derived. Ongoing investigations suggest that *Argyroxiphium*, *Calycadenia*, *Holocarpha*, *Layia*, *Wilkesia*, and other genera each exhibit distinctive substitution and accumulation tendencies. Flavonoids may be helpful in developing and testing phylogenetic hypotheses in the Madiinae.

The North American Madiinae (Asteraceae: Heliantheae) consist of about a dozen genera distributed from Baja California to British Columbia, with a major concentration of taxa in California. *Layia* Hook. & Arn. and *Madia* Molina, two of the larger genera, contribute conspicuously to spring floral displays in California. The Hawaiian Madiinae consist of three genera: *Argyroxiphium* DC. with five species, *Dubautia* Gaudich. with 21 species (33 taxa in all), and *Wilkesia* A. Gray with only two species. Arguably the most spectacular members of the island taxa are the subspecies of *A. sandwicense*: subsp. *sandwicense* grows on Mauna Kea on the island of Hawaii; subsp. *macrocephalum* (A. Gray) Meyrat, grows on Haleakala on Maui. Both of these have suffered serious predation and are maintained only with special effort. Species of *Dubautia* occur on all major islands of the archipelago. They exhibit a very wide range of morphological forms and are ecologically the most diverse within the Hawaiian tarweeds. *Wilkesia* consists of two species, *W. gymnoxiphium* A. Gray and *W. hobdyi* H. St. John, both of which are restricted to western Kauai. The former is much more common, occurring in comparatively large populations along the western edge of Waimea Canyon. *Wilkesia hobdyi* is known from only two sites on the far western ends of two ridges and consists of perhaps no more than 200 individuals; it is under severe predation from feral goats and may be on the verge of extirpation.

The Madiinae provide ideal opportunities for the study of evolutionary divergence. In a few genera, hypotheses of species formation have been proposed, and recently tests of these ideas have been conducted using electrophoretic data (Warwick & Gottlieb, 1985; Witter & Carr, 1988). However, it is surprising that in a group as well studied as the tarweeds, so few explicit hypotheses of phylogenetic relationships have been proposed. There now exists a large enough body of data that such hypotheses could be constructed, and these could serve as frameworks for assessment of congruence among different types of data and for testing models of speciation. Flavonoid data could serve in both roles. Since the genetic bases of their biosynthesis are well known (Heller, 1986), these compounds ultimately can be used to construct phylogenies and to assess phylogenies based on other data.

SURVEY AND BIOSYNTHETIC TRENDS

Before dealing with the flavonoids from a phylogenetic viewpoint, we will discuss their structural

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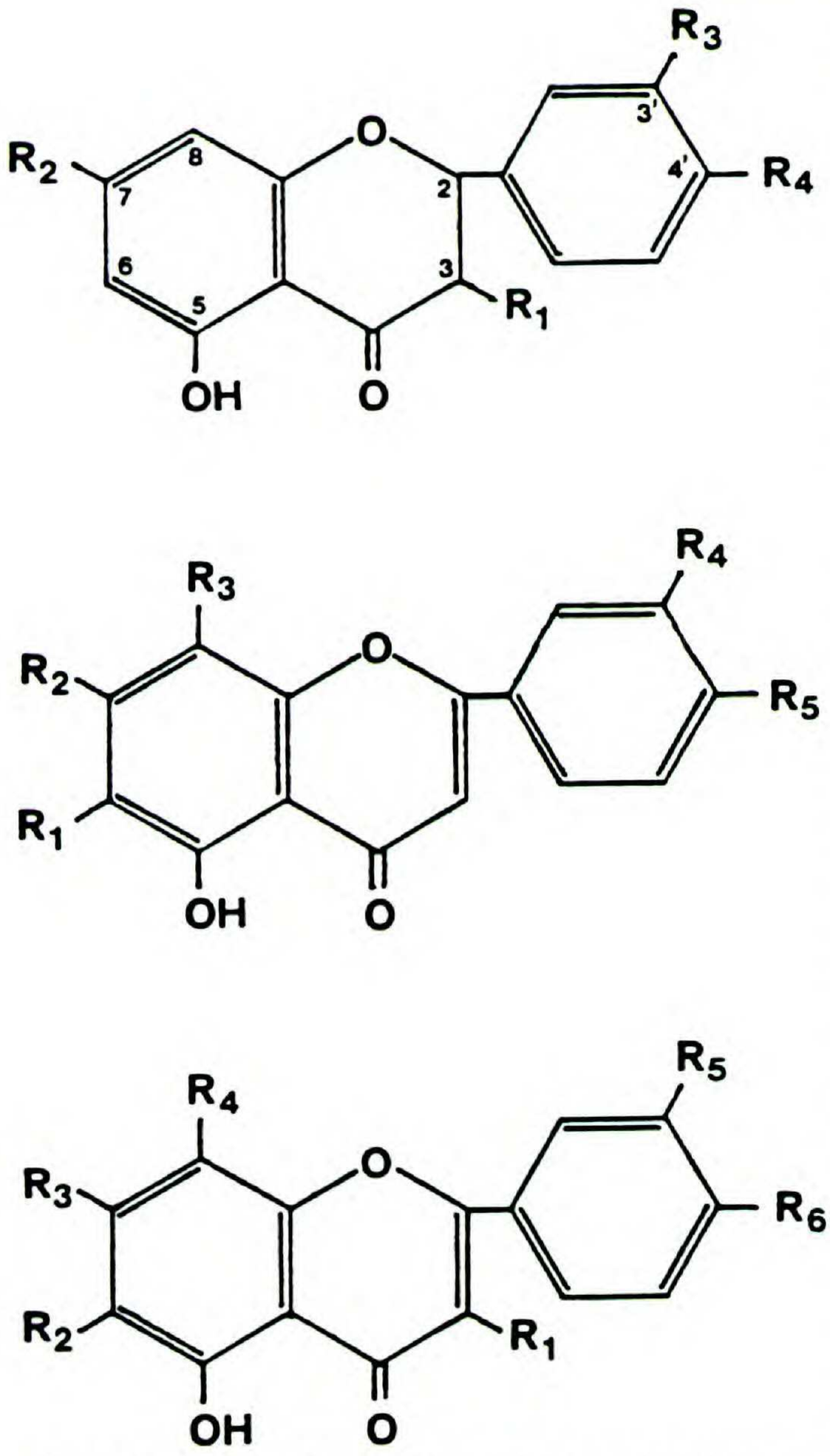


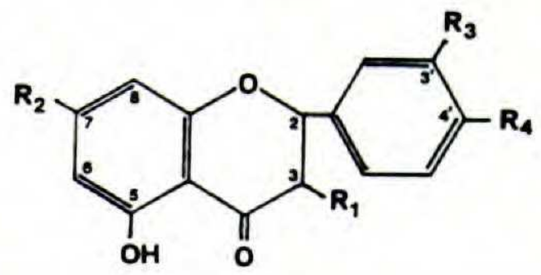
FIGURE 1. Structures of tarweed flavonoid aglycones. Upper structure represents flavanones and dihydroflavonols. Flavones and flavonols are represented by the middle and lower structures, respectively. See Tables 1–3 for substitution patterns (R = H, OH, or OMe) and occurrence of individual compounds in the tarweed genera.

variation and accumulation in the tarweeds. The variety of substitution patterns observed is based upon four flavonoid classes, flavanones, dihydroflavonols, flavones, and flavonols (Fig. 1). The glandular exudates of *Adenothamnus validus* (Brandegee) Keck and all species of *Holocarpha* (DC.) Greene contain all four classes (Crins & Bohm, 1987, 1988a, b). Some of the $n = 13$ (glandular) species of *Dubautia*, such as *D. ciliolata* (DC.) Keck, accumulate flavanones, flavones, and flavonols in their resins, and two classes (flavanones and flavonols) among their vacuolar constituents (Crins et al., 1988a). All species of *Hemizonia* DC. examined so far accumulate flavones and flavonols, and all but one of these also accumulate flavanones (Tanowitz et al., 1987). None of the taxa of *Argyroxiphium*, *Calycadenia* DC., *Lagophylla* Nutt., *Layia*, or *Wilkesia* examined so far accumulate flavanones. Taxa from these genera that do accumulate flavonoids exhibit flavones and flavonols. Two of the species of *Layia* examined, *L. carnosa* (Nutt.) T. & G. and *L. chrysanthemoides* (DC.) A. Gray, lack flavonoids externally, but both secrete large amounts of scopoletin (a coumarin) (Crins et al., 1988b). All tarweeds contain vacuolar glycosylated flavonoids.

Tarweed flavonoids exhibit a wide variety of substitution patterns. Tables 1–3 present the known arrays of flavonoid aglycones in leaf exudates. It is immediately apparent that tarweeds have a propensity for O-methylation. Another prominent feature is the abundance of extra hydroxylation at positions 6 and/or 8, with these hydroxyls frequently being methylated. These series of compounds can be examined in light of biosynthetic considerations, and some generalizations arise. O-Methylation of flavanones can occur at positions 7 and/or 4′. Dihydroflavonols may be formed from their flavanone precursors either before or after O-methylation has occurred. The same is true for later biosynthetic steps (formation of flavones from flavanones or flavonols from dihydroflavonols). However, the series of compounds within a given plant generally suggests that O-methylation at position 7 occurs early in the biosynthetic pathway and that extra hydroxylation occurs after the bond between carbons 2 and 3 has become unsaturated. We have found no evidence of 6-hydroxyflavanones or 6-hydroxydihydroflavonols. [The report of 6-hydroxyeriodictyol 7-methyl ether by Crins & Bohm (1987) must be corrected to 7-O-methyldihydroquercetin in light of new evidence on structural differentiation of flavanones and dihydroflavonols using mass spectrometry (Balza et al., 1988).] The affinities of the 6- (and 8-) O-methyltransferase enzymes for their substrates must be very high, as 6- and 8-hydroxy derivatives are only infrequently encountered.

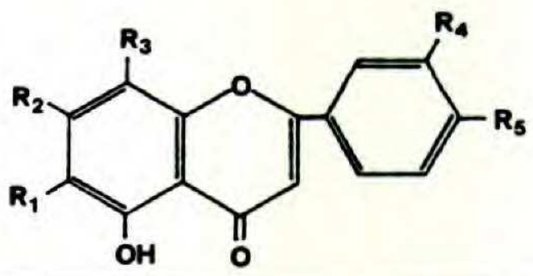
Several taxa lack external flavonoid aglycones. In *Layia chrysanthemoides*, loss of glandular structures is correlated with the inability to secrete these compounds, although the leaf wash did contain the coumarin scopoletin. In the $n = 14$ *Dubautia* taxa, glands are absent from the foliage and stem, although there may be some glands on the pedicels and corollas (Carr, 1985). In most cases insufficient flowering material was available to test for flavonoids. However, in the case of *D. scabra* (DC.) Keck, where large amounts of flowering material was available, flavonoids were not detected (Crins et al., 1988a). In several mainland

TABLE 1. Occurrence and substitution patterns of flavanones and dihydroflavonols in tarweed genera. R-groups on model structure correspond to R-groups in columns one to four. H = hydrogen; OH = hydroxyl group; OMe = methoxyl group; Ad = *Adenothamnus*; D = *Dubautia*; Hm = *Hemizonia*; Ho = *Holocarpha*; M = *Madia*. Data for *Madia* are based on an incomplete survey of *M. elegans* D. Don.



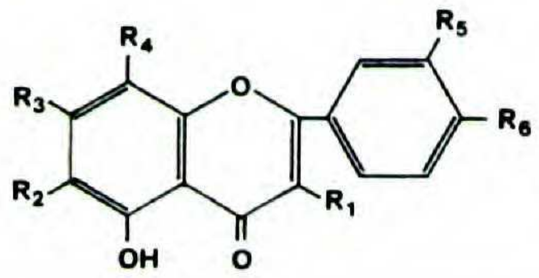
Substitution pattern				Genus				
R_1	R_2	R_3	R_4	Ad	D	Hm	Ho	M
H	OH	H	OH	+	+	+	−	−
H	OH	H	OMe	+	−	−	−	−
H	OMe	H	OH	−	+	−	−	−
H	OMe	H	OMe	+	−	−	+	−
H	OH	OH	OH	−	−	+	−	−
H	OMe	OH	OH	+	+	+	+	−
H	OMe	OH	OMe	+	−	−	−	−
OH	OMe	OH	OH	+	−	−	+	+

TABLE 2. Occurrence and substitution patterns of flavones in the tarweed genera. R-groups on model structure correspond to R-groups in columns one to five. H = hydrogen; OH = hydroxyl; OMe = methoxyl; Ad = *Adenothamnus*; Ar = *Argyroxiphium*; C = *Calycadenia*; D = *Dubautia*; Hm = *Hemizonia*; Ho = *Holocarpha*; Lg = *Lagophylla*; Ly = *Layia*; M = *Madia*; W = *Wilkesia*.



Substitution pattern					Genus									
R_1	R_2	R_3	R_4	R_5	Ad	Ar	C	D	Hm	Ho	Lg	Ly	M	W
H	OH	H	H	OH	−	+	−	+	−	+	−	+	+	−
H	OMe	H	H	OH	−	−	−	+	−	−	+	−	−	−
OH	OH	H	H	OH	−	−	−	+	−	−	−	−	−	−
OMe	OH	H	H	OH	+	−	−	+	−	−	−	+	+	−
OMe	OMe	H	H	OH	−	+	−	+	−	−	+	+	−	+
OMe	OH	H	H	OMe	−	−	−	−	−	−	−	+	−	−
H	OH	OMe	H	OH	−	−	−	+	−	−	−	−	+	+
H	OMe	OMe	H	OH	−	+	−	−	−	−	−	−	−	+
OMe	OH	OMe	H	OH	−	−	−	−	−	−	−	−	−	+
OMe	OMe	OMe	H	OH	−	+	−	−	−	−	−	−	−	+
H	OH	H	OH	OH	−	−	−	−	+	−	−	−	−	−
H	OMe	H	OH	OH	+	−	−	+	−	+	−	−	−	+
H	OH	H	OH	OMe	−	−	−	+	−	−	−	−	−	−
H	OH	OMe	OH	OH	−	−	−	−	−	−	−	−	−	+
OMe	OH	H	OH	OH	+	−	−	−	−	−	−	+	−	−
OMe	OH	H	OMe	OH	−	−	−	−	−	+	+	−	−	−
OMe	OH	H	OMe	OMe	−	−	−	−	−	−	−	+	−	−
OMe	OMe	H	OH	OH	−	−	−	+	−	−	−	−	−	+
OMe	OMe	H	OMe	OMe	−	−	−	−	−	−	+	−	−	−
OMe	OH	OH	OH	OH	−	−	−	−	+	−	−	−	−	−
OMe	OH	OMe	OH	OH	−	−	−	−	−	−	−	−	−	+
OMe	OMe	OMe	OH	OH	−	−	−	−	−	−	−	−	−	+
OMe	OMe	OMe	OMe	OH	−	−	+	−	−	−	−	−	−	−

TABLE 3. Occurrence and substitution patterns of flavonols in tarweed genera. R-groups on model structure correspond to R-groups in columns one to six. H = hydrogen; OH = hydroxyl; OMe = methoxyl; Ad = *Adenothamnus*; Ar = *Argyroxiphium*; C = *Calycadenia*; D = *Dubautia*; Hm = *Hemizonia*; Ho = *Holocarpha*; Lg = *Lagophylla*; Ly = *Layia*; M = *Madia*; W = *Wilkesia*.



Substitution pattern						Genus									
R_1	R_2	R_3	R_4	R_5	R_6	Ad	Ar	C	D	Hm	Ho	Lg	Ly	M	W
OH	H	OH	H	H	OH	−	−	−	+	−	−	−	−	−	−
OMe	H	OH	H	H	OH	+	−	−	−	−	+	−	−	−	+
OH	H	OMe	H	H	OH	−	−	−	+	−	+	−	−	−	−
OH	H	OH	H	H	OMe	−	−	−	−	+	−	−	−	−	−
OMe	OMe	OH	H	H	OH	+	−	+	−	−	−	−	+	−	−
OMe	OMe	OMe	OMe	H	OH	−	−	+	−	−	−	−	−	−	−
OH	H	OH	H	OH	OH	−	−	−	+	+	−	−	−	−	−
OMe	H	OH	H	OH	OH	−	−	−	−	+	+	−	−	+	−
OMe	H	OH	H	OMe	OH	−	−	−	−	−	+	−	−	+	−
OH	H	OMe	H	OH	OH	+	−	−	−	−	+	−	−	−	−
OMe	H	OMe	H	OH	OH	−	−	−	−	+	+	−	−	−	−
OMe	OH	OMe	H	OH	OH	−	−	−	−	−	+	−	−	−	−
OH	OMe	OH	H	OH	OH	−	−	−	−	+	−	−	−	−	−
OMe	OMe	OH	H	OH	OH	−	−	−	−	+	+	−	−	−	−
OMe	OMe	OH	H	OMe	OH	−	−	−	−	−	−	+	−	−	−
OMe	OMe	OMe	H	OH	OH	−	−	−	−	+	−	+	−	−	−
OMe	OMe	OMe	H	OH	OMe	−	−	−	−	−	−	+	−	−	−
OMe	OH	OMe	OMe	OH	OH	−	−	+	−	−	−	−	−	−	−
OMe	OMe	OH	OMe	OH	OH	−	−	−	−	+	−	−	−	−	−
OMe	OMe	OMe	OMe	OH	OH	−	−	+	−	−	−	−	−	+	+
OMe	OMe	OMe	OMe	OMe	OH	−	−	+	−	−	−	−	−	−	−
OMe	H	OH	OMe	OH	OH	−	−	−	−	+	−	−	−	−	−
OMe	H	OMe	OMe	OH	OH	−	−	+	−	+	−	−	−	−	−

tarweeds, glands are present but no flavonoid aglycones are secreted: *Achyrachaena mollis* Schauer, *Blepharipappus scaber* Hook., *Lagophylla minor* (Keck) Keck, *L. ramosissima* Nutt., and *Layia carnosa*. Coumarins were observed in some of these, however (Crins et al., 1988b). Many of these plants have highly reduced or modified floral and/or inflorescence structures compared with their congeners, or the subtribe as a whole. *Achyrachaena mollis* is clearly divergent, with its reduced, yellow-orange rays and reduced inflorescence. Within *Lagophylla*, *L. minor* has few glands, and *L. ramosissima* has greatly reduced capitula, compared with such species as *L. glandulosa* A. Gray, which yields copious flavonoid-rich resin. *Layia carnosa* is clearly differentiated from other *Layia* species by its reduced white rays and specialized habitat preference.

Some trends are beginning to emerge in terms of accumulation tendencies and substitution patterns among the ten genera for which we have data. *Adenothamnus validus*, *Hemizonia* species, and *Holocarpha* species accumulate flavanones, as do several xerophytic species of *Dubautia*: *D. arborea* (A. Gray) Keck, *D. ciliolata*, and *D. linearis* (Gaudich.) Keck subsp. *hillebrandii* (H. Mann) G. Carr. These three taxa of *Dubautia* occur on the island of Hawaii. In contrast, *D. linearis* subsp. *linearis* from Lanai, and *D. sherffiana* Fosb. from Oahu (both $n = 13$) have much less diverse arrays of external flavonoids, and they do not accumulate flavanones. This trend is further emphasized since all $n = 14$ taxa lack external flavonoids and they all occur on the older islands. Thus, flavonoid diversity parallels cytological and morphological radiation in *Dubautia* (Carr, 1985; Crins et al., 1988a; Crins & Bohm, unpublished data).

The capacity of a particular taxon to accumulate flavanones and dihydroflavonols must be used judiciously in considering relationships since these classes of compound serve as precursors of flavones and flavonols, respectively (Heller, 1986). However, if flavanones or dihydroflavonols exhibit substitution patterns that are not seen in flavones or

flavonols from the same taxon, then information of phylogenetic interest can be gained. Methylation at any hydroxylated position on the flavonoid nucleus requires a specific O-methyltransferase; a high level of site selectivity has also been shown for O-glycosyltransferases (Heller, 1986; Ibrahim et al., 1987). Assuming that such enzyme specificity is general in plants, relationships of taxa within genera and perhaps among genera in the Madiinae can be examined.

In *Adenothamnus* and *Holocarpha*, one flavanone (7,4′-dimethylnaringenin), present in large quantities, has no counterpart among suites of flavones and flavonols. Thus it appears that the 4′-O-methylation step is unrelated to subsequent biosynthetic steps, and this flavanone is an accumulation product in its own right. This is also true of 4′-methylnaringenin and persicogenin in *Adenothamnus*.

On the generic level some common tendencies in substitution patterns are apparent among flavones and flavonols. *Argyroxiphium* appears to produce only flavones, but only one species has been examined. Four genera have 3-unsubstituted flavonols in their pigment profiles, *Adenothamnus*, *Dubautia*, *Hemizonia*, and *Holocarpha*. Of this group only *Dubautia* lacks the corresponding 3-O-methyl derivatives. All genera are capable of 7-O-methylation although each genus also contains compounds in which the 7-hydroxyl group remains unsubstituted. All genera produce at least one 6-O-methylated compound. Less common is 8-O-methylation, which was observed in *Argyroxiphium*, *Calycadenia*, *Dubautia*, *Hemizonia*, *Madia*, and *Wilkesia*. Within the Madiinae as a whole there is a strong tendency for the hydroxyl groups at positions 6 and/or 8 to be methylated. Only three of ten genera studied exhibit 6-hydroxyflavonoids (*Calycadenia*, *Dubautia*, and *Holocarpha*), and only *Hemizonia* contains 8-hydroxyflavonoids. B-Ring O-methylation of flavones and flavonols is also restricted in distribution: 3′-O-methylation is seen in *Calycadenia*, *Holocarpha*, *Lagophylla*, *Layia*, and *Madia*, and 4′-O-methylation is known only in *Dubautia*, *Hemizonia*, *Lagophylla*, and *Layia*. *Lagophylla glandulosa* and *Layia hieracioides* (DC.) Hook. & Arn. are alone in exhibiting 3′,4′-di-O-methylation (Crins et al., 1988b).

Evolutionary Trends

Analysis of Hybrids in *Dubautia*

We begin our discussion of the application of flavonoid data to tarweed systematics by examining a case of natural hybridization between two species of *Dubautia*. On the island of Hawaii, lava flows from two of its major volcanoes, Mauna Loa and Mauna Kea, meet in an area known as the "Saddle." *Dubautia scabra* ($n = 14$) and *D. ciliolata* ($n = 13$) co-occur here. *Dubautia scabra* exhibits very high chromosomal homology with all of the $n = 13$ species and is considered to be their nearest living ancestor (Carr, 1985). Natural hybrids have developed in areas of marginal or intermediate habitat in the contact zone. Carr & Kyhos (1981) suggested that these hybrids were generally F_1s with a few higher-generation recombinants perhaps also present.

Flavonoid data clarify the nature of the hybrid population. The flavonoid glycosides of the parental species differ, with *D. ciliolata* producing kaempferol and quercetin 3-O-monoglucosides and 3-O-rhamnosylgalactosides and *D. scabra* producing quercetin 3-O-glucoside and 6-O-methoxyflavonol mono- and diglycosides. The hybrids share 3-O-monoglucosides with both parents, but all of their diglycosides are "hybrid" compounds, with rhamnose being derived from *D. ciliolata* and glucose from *D. scabra* (Crins et al., 1988a). All of the hybrid plants contain the same array of glycosides.

Glandular resins allow further resolution of the structure of the hybrid population. *Dubautia scabra* does not produce external flavonoids; the resin of *D. ciliolata* contains several flavones, two flavanones, and one flavonol. Quercetin and kaempferol appear to be restricted to the hybrids possibly on account of recombination or other disruption of biosynthesis.

The varying distributions of compounds among hybrid plants indicate differing degrees of disruption of biosynthetic pathways, which suggests the presence of higher-generation recombinants. When considering the entire set of hybrids in the sample, gradation in the complexity of flavonoid aglycone arrays emerges. The field-collected hybrids show considerable variation in their arrays. However, synthetic F_1 hybrids produced under controlled conditions were chemically uniform. Comparison of the flavonoid chemistry of the synthetic and natural hybrids strongly suggests that all of the natural hybrids are at least second-generation crosses, and the complexities of some of their profiles suggests introgression toward *D. ciliolata*.

Population Analysis in *Calycadenia*

Resin flavonoids have also served usefully as markers of inter- and intrapopulational differences in *Calycadenia ciliosa* Greene (Emerson et al., 1986). *Calycadenia ciliosa* consists of at least five structurally distinct chromosome races (Carr &

Carr, 1983), and high degrees of structural heterozygosity in some populations complicate the situation. The Dry Creek race is the most variable morphologically, the most widespread geographically (Carr & Carr, 1983), and the most diverse chemically (Emerson et al., 1986). In fact, there is a striking correlation among morphological variability, geographical extent, and chemical diversity in all races of *C. ciliosa*. For the most part, flavonoid data concur with the cytogenetic results. However, it seems that the Dry Creek race is most likely to be the primitive race, rather than Ciliosa, as concluded earlier (Emerson et al., 1986), since the former has the most complex array of flavonoids, including the only compound with a monohydroxylated B-ring. The Lewiston and Corning races both accumulate 5,3′,4′-trihydroxy-3,6,7,8-tetramethoxyflavone along with one of its precursors unsubstituted at position 8. None of the precursors remain in the Ciliosa and Pillsbury races, suggesting increased efficiency in substrate utilization.

FLAVONOID PATTERNS WITHIN *DUBAUTIA*

Several interesting evolutionary problems can be found in the genus *Dubautia*, whose 21 species (33 taxa in all) are ordered into three sections. Section *Dubautia* consists of ten species (18 taxa) and is characterized by having $n = 14$ and, relevant to our interests, being eglandular and thus lacking resinous flavonoids. Section *Venoso-reticulatae* (A. Gray) G. Carr consists of only *D. latifolia* (A. Gray) Keck which has $n = 14$, is eglandular, and, interestingly, is a liana. The remaining ten species (14 taxa) comprise sect. *Railliardia*, which is heterogeneous with regard to chromosome number and leaf surface chemistry. All taxa in this last section have $n = 13$ except *D. scabra*, which has $n = 14$ in common with the other two sections. It also lacks leaf surface flavonoids in common with the other two sections. *Dubautia scabra* is considered to be the nearest extant relative to the $n = 13$ species, as indicated by enzyme electrophoretic data (Witter & Carr, 1988). The absence of flavonoid aglycones in *Dubautia herbstobatae* is more difficult to explain. It may have resulted from a reversal to a nonglandular state, or this may be a more primitive taxon than has previously been suspected. *Dubautia herbstobatae* might have been the first offshoot of the $n = 13$ lineage, before glandularity and flavonoid secretion became prominent features of the group, a contention supported by its occurrence on Oahu, the oldest island, on which $n = 13$ species presently occur (Fig. 2).

Within the $n = 13$ glandular species it is possible to distinguish two groups based on comparative complexity of flavonoid profiles. *Dubautia linearis* subsp. *linearis*, *D. reticulata* (Sherff) Keck, and *D. sherffiana* have simple aglycone profiles consisting of three compounds each. A close relationship between *D. linearis* subsp. *linearis* and *D. sherffiana* is suggested by their identical profiles. This is the only case in *Dubautia* where profiles of two different taxa were identical. The other three species in the $n = 13$ glandular group that we have examined, *D. arborea*, *D. ciliolata* subsp. *glutinosa* G. Carr, and *D. linearis* subsp. *hillebrandii*, exhibit much more complex arrays of flavonoids in their resins. Each of these latter three taxa has its own peculiar profile. One of the more interesting things to emerge from flavonoid analysis of this group of taxa is the dissimilarity between these two subspecies of *D. linearis*. *Dubautia linearis* subsp. *linearis* occurs on Maui and Lanai (but not on Hawaii), while *D. linearis* subsp. *hillebrandii* occurs on the island of Hawaii. (*Dubautia linearis* subsp. *opposita* (Sherff) G. Carr) occurs on Molokai and was not available for study.) *Dubautia linearis* subsp. *linearis* has a flavonoid pattern identical to that of *D. sherffiana*. This relationship is supported by close similarities in vegetative anatomy (Carlquist, 1959) and morphology (Carr, 1985). *Dubautia linearis* subsp. *hillebrandii* differs substantially from subsp. *linearis* and may be more closely related to other taxa from the islands of Maui and Hawaii, contrary to its current placement in *D. linearis*. In fact, Carlquist (1959) pointed out the anatomical and morphological similarities of subsp. *hillebrandii* with *D. arborea* and *D. menziesii* (A. Gray) Keck. Furthermore, Witter & Carr (1988) showed that subsp. *hillebrandii* had higher genetic identities with *D. reticulata*, *D. dolosa* (Degener & Sherff) G. Carr, *D. platyphylla* (A. Gray) Keck, *D. menziesii*, and *D. arborea* than it did with *D. sherffiana*. (Data for *D. linearis* subsp. *linearis* were not available for comparison.) Further studies of this system will be necessary to gain a better understanding of relationships.

Flavonoid glycoside patterns may likewise provide indications of relationships within *Dubautia*. On a relatively coarse scale, the presence of an eriodictyol glycoside correlates well with the xerophytic habit, although at least two mesophytes (*D. laxa* Hook. & Arn. subsp. *hirsuta* (Hillebrand) G. Carr and *D. microcephala* Skottsb.) also contain this compound. On the other hand, several of the mesophytes (*D. knudsenii* Hillebrand subsp. *knudsenii*, *D. laevigata* A. Gray, *D. latifolia* (A. Gray)

Keck, *D. plantaginea* Gaudich. subsp. *plantaginea*, and *D. raillardioides* Hillebrand) lack this compound. It is unclear whether the occurrence of this compound signifies relationship, or whether this is a case of parallelism (see discussions of *Hemizonia* and *Holocarpha* below), since the suggested relationships of these mesophytes to the xerophytes do not conform to those presented in the scheme of Witter & Carr (1988).

MULTIVARIATE ANALYSIS OF *HEMIZONIA* FLAVONOIDS

Tanowitz and associates (Proksch et al., 1984; Tanowitz et al., 1987) have examined the flavonoid aglycones of several species of *Hemizonia*. In their work we can also look for indications of relationships at the macroevolutionary level. They attempted to elucidate relationships by comparing the flavonoid aglycone arrays of 18 taxa of *Hemizonia*, including three perennials (*H. greeneana* Palmer subsp. *peninsularis* Moran, *H. minthornii* Jepson, and *H. streetsii* A. Gray) using phenetic methods. Their ordination and clustering results suggest that each of these taxa belongs to a different subgroup within a heterogeneous assemblage of taxa, including *H. fitchii* A. Gray of sect. *Centromadia* Keck, and an assortment of species from sect. *Madiomeris* Nutt as emended by Tanowitz. The cluster analysis also split sect. *Madiomeris* into two groups.

If we consider the phenetic relationships within sect. *Madiomeris* in a flavonoid biosynthetic context, several contrasts are evident. Before proceeding it is important to remember that the presence of a compound in a profile requires that all precursors in its pathway must have been present at some time so that, although the absence of a compound will affect the computation of distance measures, it will not necessarily affect an estimate of relationship based on biosynthetic principles.

After recoding the data to account for precursors (only unique end products are useful for phylogenetic analysis) there are only six compounds that can potentially yield useful phylogenetic information and one additional compound that serves as an autapomorphy. When this data set is analyzed using Penny's branch-and-bound method to determine the most parsimonious trees (using PHYLIP), two topologies are found. (Since a few taxa from this section are not included in the data set owing to lack of flavonoid data, the group analyzed is paraphyletic.) Nevertheless, the topologies provide novel frameworks for testing ideas about relationships within the section. Figure 3 illustrates the topologies and a consensus tree based on them.

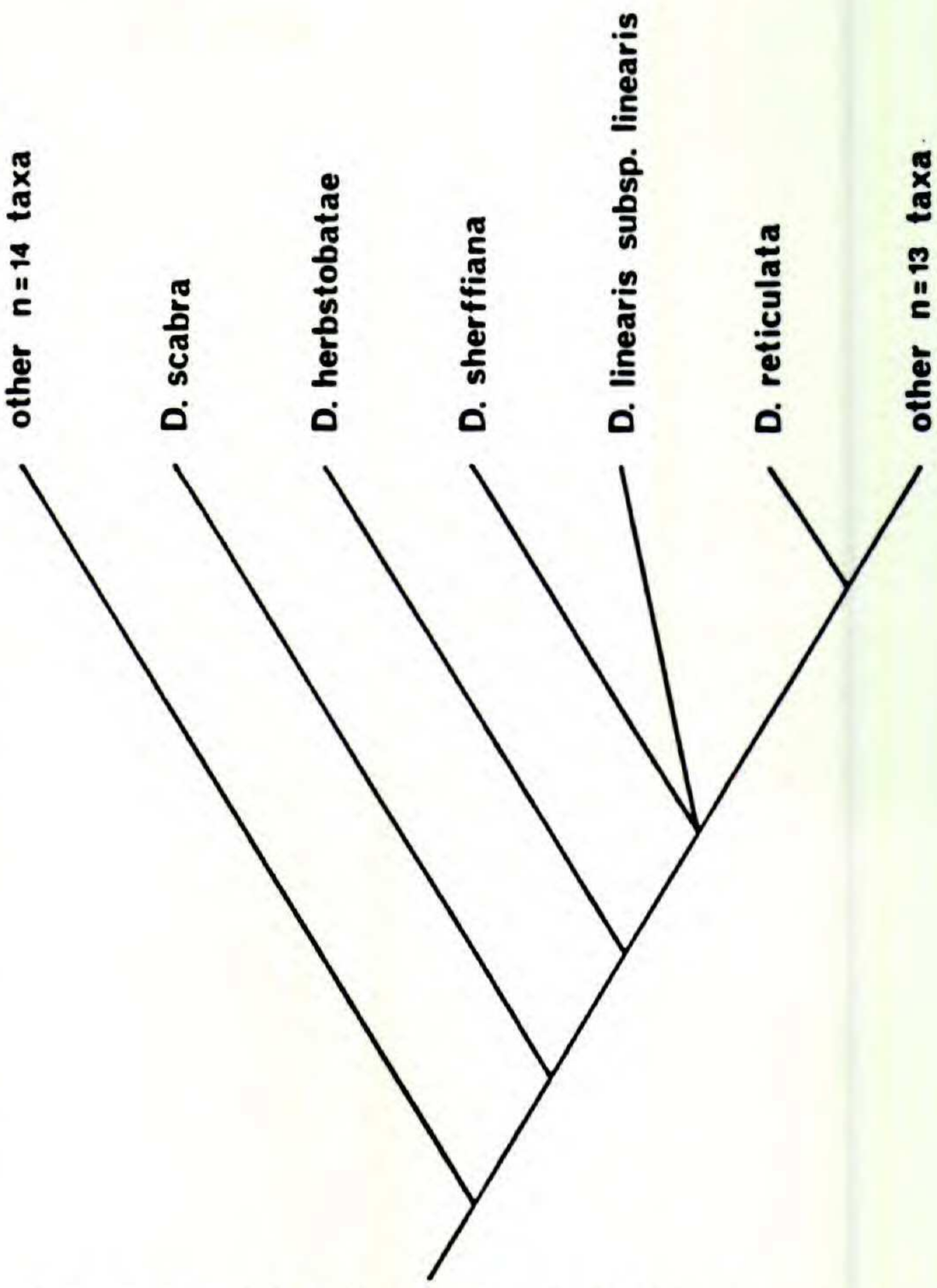


FIGURE 2. Partial resolution of phylogenetic relationships within *Dubautia* sect. *Railliardia*, based on flavonoid aglycones.

Although the consensus tree is very poorly resolved, it suggests that two subgroups exist within sect. *Madiomeris* (excluding the perennials). These subgroups are not entirely consistent with the groupings found in the phenetic analyses reported by Tanowitz et al. (1987). In their group II, *H. halliana* Keck is included with *H. arida* Keck, *H. fasciculata* (DC.) T. & G., *H. pallida* Keck, and *H. paniculata*. *Hemizonia pentactis* (Keck) Keck is placed in their group III. The group placements of *H. halliana* and *H. pentactis* are reversed in our analysis relative to their positions in the phenetic analysis of Tanowitz et al. (1987).

When the data for the three perennials are included in the analysis, almost 1,500 most parsimonious trees are possible. It has not been possible to examine more than a small number of these trees, but a few features are worthy of note. In the sample of 50 trees examined, *H. arida*, *H. fasciculata*, *H. pallida*, *H. paniculata*, and *H. pentactis* are always maintained as a group. However, the distinction between these five taxa and the other group found when perennials were excluded (consensus tree, Fig. 3) is not always upheld. Little can be said about the relationships of the perennials, except that *H. streetsii* is always grouped with *H. lobbii* in the 50 trees. Thus, the flavonoid

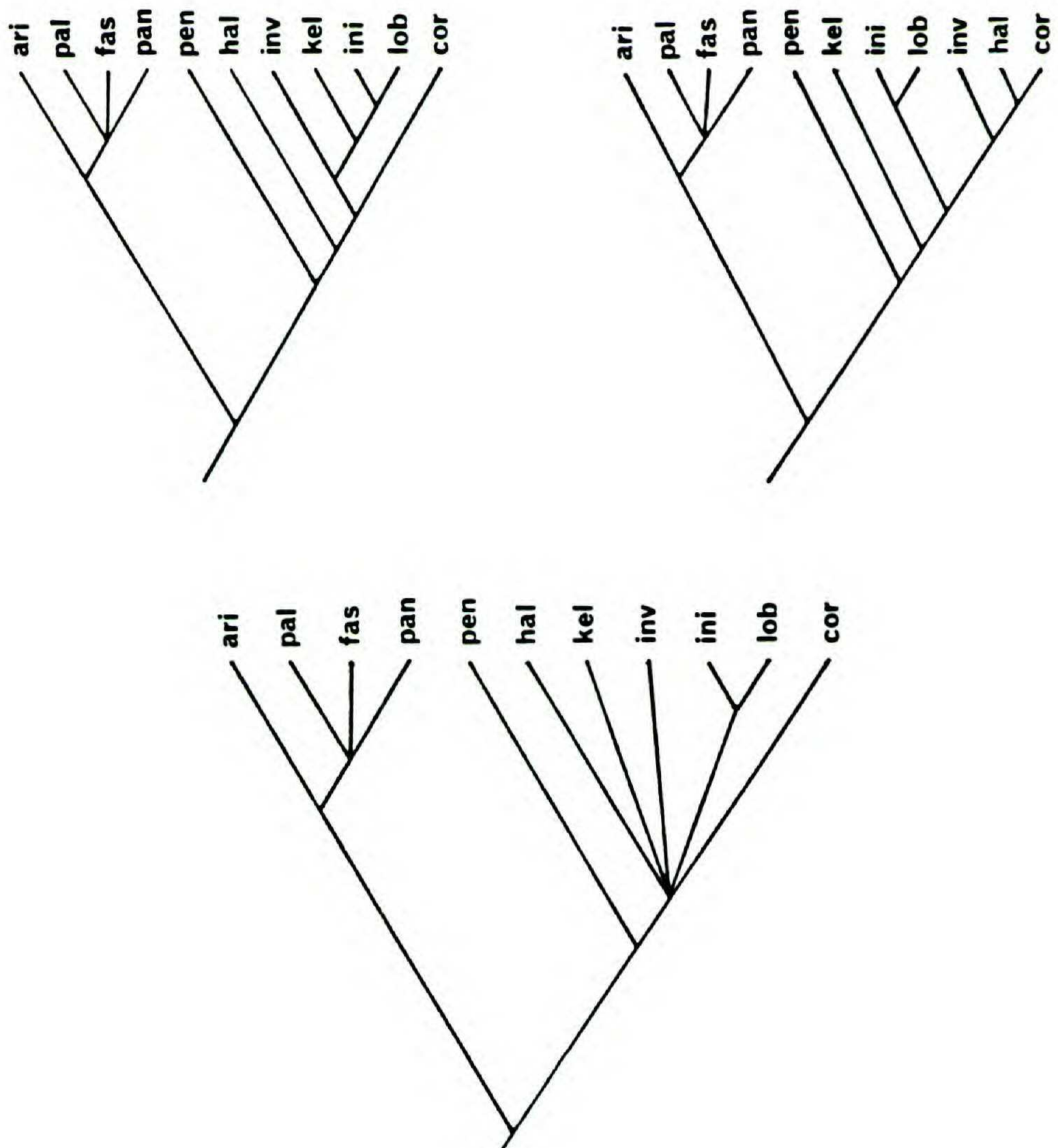


FIGURE 3. Alternative phylogenetic trees of the taxa of *Hemizonia* sect. *Madiomeris* for which flavonoid aglycone data are available, and their consensus tree. Key to species names: Ari = *H. arida*; Con = *H. conjugens*; Cor = *H. corymbosa*; Fas = *H. fasciculata*; Hal = *H. halliana*; Inc = *H. increscens*; Kel = *H. kelloggii*; Lob = *H. lobbii*; Pal = *H. pallida*; Pan = *H. paniculata*; Pen = *H. pentactis*.

data provide general indications of relationship within sect. *Madiomeris*, but fine-scale resolution does not seem possible.

PHYLOGENETIC ANALYSIS OF *HOLOCARPHA* USING FLAVONOIDS

Whenever possible, an attempt should be made to integrate all available information into a classification scheme or a phylogenetic hypothesis. In a sense, this has been done with *Hemizonia*, where a recently revised genus has been studied in terms of its flavonoid chemistry, and further suggestions about relationships have been set forth. However, data are incomplete for many taxa, and no explicit phylogeny for the genus has been proposed against which the chemical data could be examined. Since there is no consensus about the way in which chemical changes occur during the process of evolution (Gornall & Bohm, 1978), a test of congruence of chemical data against a phylogeny derived from other sources (morphology, cytology, etc.) would be more prudent. In the case of *Holocarpha*, a genus of four summer-flowering, self-incompatible annuals, we generated several alternative cladograms using various algorithms (PHYLIP version 2.8, Felsenstein, 1985). Three cladograms were based on 12 discrete-state morphological characters, one 'cladogram' was based on a scenario where two independent aneuploid events were hypothesized. The most parsimonious cladogram for the morphological data set was supported by four different algorithms (Crins & Bohm, 1988b). Flavonoid aglycone biosynthetic data were then superimposed on these topologies.

Fourteen compounds have been characterized from species of *Holocarpha*. The presence or absence of terminal steps in the biosynthetic network are more or less species-specific. For example, the capacity to hydroxylate position 6 in 3,7-di-O-methylquercetin is lost in *H. macradenia* (DC.) Greene, and only *H. heermannii* (Greene) Keck can dehydrogenate the 2-3 bond and subsequently methylate the hydroxyl group at carbon-3.

The three cladograms yield similar results in terms of their congruence with the flavonoid data. All three require parallel losses of the capacity to produce 7-O-methylluteolin in *H. heermannii* and *H. macradenia*, and 3-O-methylquercetin and 3,3′-di-O-methylquercetin in *H. obconica* (Clausen & Keck) Keck and *H. macradenia*. All other losses or gains in biosynthetic capacity are autapomorphies. The least parsimonious tree for morphological characters requires only a single parallelism, for the loss of 7-O-methylluteolin. The loss of methylated quercetin derivatives is a synapomorphy uniting *H. macradenia* and *H. obconica* on this tree. The tree most consistent with the chemical data is the least parsimonious on morphological and cytological grounds (Crins & Bohm, 1988b). It should be noted that the difference (based on flavonoid data) is a single step, however. In the present case, where morphological, cytological, and ecological (Palmer, 1982) data all support a parsimonious hypothesis of evolution, we must suggest that flavonoid aglycone biosynthesis has undergone some degree of parallelism in terms of losses in different lineages. We cannot ignore the bulk of evidence in favor of a more parsimonious interpretation of chemical changes.

SOME OBSERVATIONS ON *LAYIA*

In *Layia*, Clausen et al. (1941) defined several species on morphological and cytological grounds. Its 15 species are considered to have diverged through specialization of peripheral populations on novel substrates or in new habitats (geographical speciation). Warwick & Gottlieb (1985) obtained evidence from enzyme electrophoretic studies that supported the geographical speciation hypothesis for the species with $n = 7$. Flavonoid aglycones have also provided some support for this hypothesis. The identical nonpolar phenolic profile of *L. platyglossa* (F. & M.) A. Gray ($n = 7$) and *L. glandulosa* (Hook.) Hook. & Arn. ($n = 8$) suggests that they are close relatives, as did the hybridization experiments of Clausen et al. (1941). This evidence suggests aneuploidy with little subsequent differentiation in the genome.

Although more taxa must be examined, a pattern is emerging in which flavonoid aglycone diversity increases from total absence in *L. chrysanthemoides* ($n = 7$) to the profiles of *L. glandulosa* and *L. platyglossa* ($n = 7$), which contain a small number of compounds, to the richer profiles of the *L. hieracioides* ($n = 8$)–*L. paniculata* Keck ($n = 16$) group. *Layia septentrionalis* Keck ($n = 8$) is an outlier in that it lacks the flavonol aglycone biosynthetic pathway, and *L. carnosa* ($n = 8$) is also an outlier, lacking flavonoid aglycones entirely. It seems that as each derivative species arose, independent chemical changes occurred. There is an overall trend toward increasing complexity in the flavonoid aglycone composition of the resin, assuming that $n = 7$ is the basic chromosome number of the genus (Gottlieb, 1987), but individual offshoots (*L. paniculata*, *L. septentrionalis*, *L. carnosa*) have taken different, occasionally divergent, routes in the process. As more species are examined, the flavonoid data should facilitate our understanding of the phylogenetic history of the genus.

FUTURE DIRECTIONS

Studies of flavonoid occurrence and variation within the tarweeds have begun to provide new insights into the modes and directions of evolution in this diverse group. Some of the numerous problems requiring attention have been mentioned above. Phylogenetic hypotheses, especially for the larger genera such as *Calycadenia*, *Hemizonia*, and *Madia* can serve as templates for the assessment of congruence with chemical and other data sets. There are numerous taxa in these and other genera for which no flavonoid or other chemical data are available. Table 4 summarizes the current state of the flavonoid surveys. We have concentrated largely on flavonoid aglycones, but our examination of flavonoid glycosides in two species of *Dubautia* and their natural hybrids indicates that these polar compounds will also be useful in tracking microevolution (Crins et al., 1988a). Preliminary chromatographic examinations of glycosides in a wide range of tarweed taxa reveal substantial differences in the occurrence of compounds that may be useful in macroevolutionary studies. These sorts of analyses provide a feedback mechanism whereby we learn more about the scale at which different types of data are useful in tracking evolution. In some cases, flavonoid suites suggest that biosynthetic parallelisms have occurred (e.g., *Hemizonia* and *Holocarpha*); in other cases, losses or gains in biosynthetic capability appear to be species-specific (e.g., *Holocarpha* and *Layia*); and in still others, flavonoids serve as accurate markers of population differentiation (e.g., *Calycadenia*).

Little is known of the functional significance of flavonoids in tarweeds or in most other groups of plants. Because of the rich and variable arrays of compounds produced by different species of tarweeds, these plants may be useful for studying flavonoids as ultraviolet radiation shields, as anti-

TABLE 4. Summary of progress in tarweed flavonoid survey. 2D TLC = chromatographic evidence for flavonoid glycosides. +/− = some species produce exudate flavonoids, some do not. The "?" indicates that no data exist at the moment.

Genera	Total numbers of species	Numbers of species examined	Flavonoid glycosides	Flavonoid aglycones	Sources of data
Achyrachaena	1	1	+ (2D TLC)	none	Crins & Bohm (unpublished data)
Adenothamnus	1	1	+	+	Crins & Bohm (1988a)
Argyroxiphium	5	2	+	+	Bohm & Fong (unpublished data)
Blepharippapus	1	1	+ (2D TLC)	none	Crins & Bohm (unpublished data)
Blepharizonia	1	0	?	?	
Calycadenia	11	5	+	+	Emerson et al. (1986)
Dubautia	21	18	+	+/−	Crins et al. (1988a)
Hemizonia	32	17	?	+	Proksch et al. (1984); Tanowitz et al. (1987)
Holocarpha	4	4	?	+	Crins & Bohm (1987); Crins & Bohm (unpublished data)
Holozonia	1	0	?	?	
Lagophylla	5	3	?	+/−	Crins & Bohm (unpublished data)
Layia	15	7	+ (2D TLC)	+/−	Crins et al. (1988b)
Madia	20	8	?	+	Bohm et al. (unpublished data)
Raillardella	5	1	+ (2D TLC)	none	Crins & Bohm (unpublished data)
Wilkesia	2	2	?	+	Bohm & Fong (unpublished data)

herbivore defense compounds, or as factors in the floral biology of the tarweeds. For example, *Dubautia* contains species in virtually all of the available habitats in the Hawaiian Islands, and flavonoid aglycone diversity is correlated with habitat as well as the age of individual islands. This system might provide an excellent opportunity to examine the relative requirements for UV screening, with species in mesic habitats presumably requiring far less protection than species in xeric environments. The relative effectiveness of different flavonoids in UV screening is unknown.

Experiments addressing the question of why such diverse arrays of compounds are produced by some species are also needed. Any study of coevolution that attempts to incorporate the role of flavonoids will require knowledge of the organisms with which the tarweeds interact. Flavonoids (at least aglycones) probably contribute to antiherbivore defenses, just as other secreted natural products, such as benzofurans and chromenes, do (Proksch & Rodriguez, 1983), but no conclusive experimental evidence is available. There is also a lack of data about natural herbivores, parasites, and pathogens of tarweeds.

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